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Clearance of *Escherichia coli* O157:H7 infection in calves by rectal administration of bovine lactoferrin.

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INTRODUCTION

Enterohemorrhagic *Escherichia coli* (EHEC) is a major food-borne pathogen causing severe disease in humans worldwide. The main reservoirs of EHEC are ruminants, mostly cattle, which harbour the bacteria in the gastrointestinal tract without causing apparent illness. Nowadays, the specific mechanisms responsible for the persistence of EHEC in ruminants are unknown and there is no effective strategy available to combat this pathogen. In this study, the treatment of EHEC infected calves with lactoferrin (LF) was investigated as a possible future strategy for reducing shedding of EHEC by ruminants.

MATERIALS AND METHODS

Nine calves were orally inoculated at the age of 12 weeks and re-inoculated 7 days later with 10^{10} CFU *E. coli* (strain NCTC12900) for 2 consecutive days. On day 4, 3 calves were orally administered 3 g lactoferrin per day for 51 days (oral treatment group) and 3 calves were rectally treated with 300 mg lactoferrin per day for 33 days (rectal treatment group). 3 calves served as controls and were sham-treated with PBS orally. Faecal excretion was monitored 2 times a week by direct plating and an enrichment. To determine the presence of bacteria in the intestinal tract after LF treatment, intestinal contents and tissues (jejunum, ileum, caecum, colon and rectum) were collected from all animals at euthanasia and were immediately tested by direct plating.

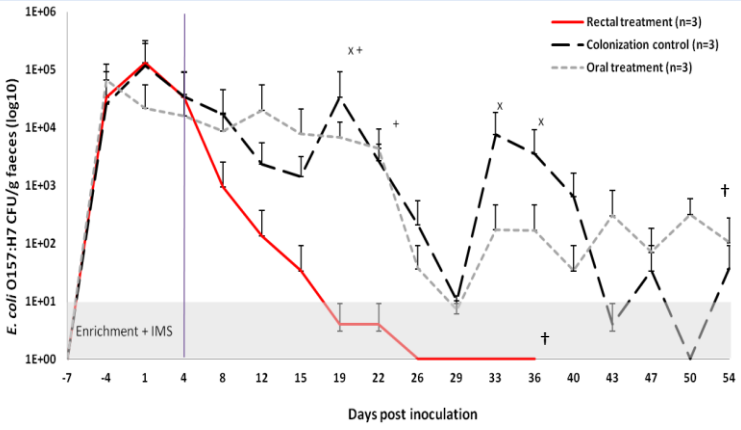

Fig.1 Average *E. coli* O157:H7 excretion following infection, oral and rectal LF treatment of calves. **x** Significant differences in bacteria excretion between control and rectal treatment groups, + Significant differences in bacteria excretion between oral and rectal treatment groups, † Euthanasia.

RESULTS

Magnitude and duration of faecal O157:H7 shedding (Fig.1) was similar for the oral treatment group and the control group. Animals in both groups were excreting bacteria in the faeces during at least 54 days. In contrast, rectal administration of LF induced a reduction in faecal *E. coli* O157:H7 excretion from day 4 onwards. The shedding pattern of the rectal treatment group and the control group was significantly different from days 19 to 22 and days 33 to 36 ($p \leq 0.05$). On day 12, two animals from the rectal treatment group already ceased excreting *E. coli* O157:H7. Both stayed negative till the end of the experiment (day 36) without further LF administration, even though the other animal in this group remained positive by direct enumeration until day 26. Faecal contents and tissues of the rectal treated group contained no bacteria, suggesting that rectal LF treatment completely eliminated the *E. coli* O157:H7 infection.

	Number of *E. coli* O157:H7 per gram intestinal content				
Lactoferrin treatment	jejunum	ileum	caecum	colon	rectum
Rectal treatment	0*	0	0	0	0
	0	0	0	0	0
	0	0	0	0	0
Control	0	0	0	0	1,00E+01
	0	0	0	0	0
	1,00E+02	6,00E+02	0	0	1,00E+02
Oral treatment	0	0	0	0	1,00E+01
	0	0	0	0	0
	0	0	2,00E+03	1,10E+03	3,00E+02

	Number of *E. coli* O157:H7 on tissue					
Lactoferrin treatment	jejunum PP**	jejunum	ileum PP	ileum	colon	recto-anal junction
Rectal treatment	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	0	0
Control	0	0	0	0	0	0
	0	0	0	0	0	1,00E+02
	0	0	0	0	0	0
Oral treatment	0	0	0	4,00E+02	0	0
	0	0	0	0	0	0
	0	0	0	3,00E+02	0	1,00E+02

*<10 colonies per gram, **pp = Peyer's patches;

CONCLUSIONS

Rectal administration of lactoferrin reduced *E. coli* O157:H7 shedding. Moreover, administration of this antimicrobial protein resulted in clearance of rectal colonization. Lactoferrin could be an interesting tool to reduce the number of cattle excreting *E. coli* O157:H7 and could thereby reduce the zoonotic risk for humans.